Pertanika J. Trop. Agric. Sci. **32(1)**: 51 - 55 (2009)

ISSN: 1511-3701

Octacosanoic Acid, Long Chains Saturated Fatty Acid from the Marine Sponges *Xestospongia* sp.

Habsah Mohamad[1*], Wan Ainur Najmiah Wan Abdul Jamil[1], Faridah Abas[2], Khamsah Suryati Mohamad[1] and Abdul Manaf Ali[3]

[1]Department of Chemistry, Faculty of Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

[2]Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia,

[3]Faculty of Agrobiotechnology, Universiti Darul Iman Malaysia, 20400 Kuala Terengganu, Terengganu, Malaysia

[*]E-mail: habsah@umt.edu.my

ABSTRACT

Octacosanoic acid has been isolated from the marine sponges *Xestospongia* sp. collected from Bidong Island, Terengganu. The structure was elucidated using various spectroscopic techniques (IR, MS, ^{1}H NMR and ^{13}C NMR). The structure and bioactivities of this long chains saturated fatty acid compound were reviewed.

Keywords: Marine sponges, *Xestospongia* sp., Bidong Islands, bioactivities, long chains saturated fatty acid

INTRODUCTION

Fatty acids are widely distributed in nature and are important as nutritional substances and metabolites in living organisms. In marine organisms, particularly sponges, fatty acids have provided some of the most interesting structural varieties. Many of these marine fatty acids originate from unusual biosynthetic pathways. In recent years, excellent reviews have appeared on the fatty acid structural types which are present in these organisms, their possible roles in membranes, and their biogenesis. However, little is known about or has been reviewed on the biomedical potential of these unusual sponge fatty acids, specifically about the differences which exist in their bioactivity, as compared to what have been reported for more common fatty acids (Carballeira, 2008).

In addition to their wide range of natural product contents, sponges turn out to be a rich source of unusual lipids which play a primary structural and functional role in their plasma membranes. Since the early studies by Litchfield *et al.* (1976), several investigations have proven the presence of long-chain, unsaturated carboxylic acids in a variety of sponges from different marine environments, all of which are members of the Demospongiae (Thiel *et al.*, 1999). These compounds display the characteristic of unsaturated patterns and may in some cases, exhibit terminal as well as mid-chain branching. They may occur as mono-, di- and trienoic compounds (tetraenoic and pentaenoic exceptionally) and cover a relatively broad carbon-number range, typically between C_{24} and C_{30}. Other organisms are apparently lacking these characteristic compounds which have thus been introduced as "demospongic" acids into the literature (Thiel *et al.*, 1999).

The total FA content of a sponge is the sum of FA of the own sponge cells and symbiont bacteria, cynobacteria and/or algal species. As a result, the total mixture of sponge FA is very complex, while the number of components

Received: 20 May 2008
Accepted: 8 October 2008
[*]Corresponding Author

exceeds 100. A lot of sponge FA is specific for the symbiont types in the sponge tissue. Some lipids isolated from marine sponges showed a cytotoxic and anti-cancer activity; therefore, these sponges may be a good source of bioactive compounds with a unique chemical structure (Imbs and Rodkina, 2004). This study reports on the isolation and structure elucidation of the new fatty acid metabolites and its bioassays.

MATERIALS AND METHODS

Sample Preparation and Extraction

Samples of *Xestospongia* sp. were collected *via* SCUBA diving at the depth of 5-10 meters off Kapas Island, in Terengganu. The sponges were directly frozen after the collection and transferred into a –20°C freezer for storage. One part of the fresh sample was preserved in methanol as a taxonomic voucher. Specimens were also deposited at the Biodiversity Museum, Oceanography Institute, Universiti Malaysia Terengganu (Voucer speciman no: B01/002/04). The remaining sponges were cleaned, chopped and dried in an air grafied oven (40°C) prior to extraction. The dried sponges were macerated in methanol 99.7%. The extracts were filtered and dried under reduced pressure using a rotary evaporator. After that these dried extracts were de-salted and kept under –20°C prior to analysis.

Isolation

The concentrated hexane extract was chromatographed over silica gel with hexane/$CHCl_3$/EtOAc to give the oily cream fractions. Further purification of the fractions by repeated colum chromatography and recrystalized from methanol yielded compound **1** (30mg, fraction 12-17 from B01/002/04).

Free Radical-Scavenging Activity (1, 1-diphenyl-2-picrylhydrazyl)

The scavenging activity of DPPH free radical of 1 was done according to the method reported by Oktay *et al.* (2003) with some modifications. The compound (50 µl) was mixed with 1.95 ml of 0.1 mM DPPH-methanol solution. Methanol was used as a blank sample in this experiment. After 30 min. of incubation at room temperature in the dark, the reduction of the DPPH free radical was measured by reading the UV absorbance at 517 nm. Butylated hydroxyanisole (BHA) and Quercetin were used as positive controls. In this study, the scavenging activity (%) was calculated using the following equation:

Free radical scavenging activity (%) = $\{(abs_{control} - abs_{sample})/abs_{control}\} \times 100$.

The scavenging activity (SA) of each test sample was classified into four ranks as follows: strongly active (SA≥70%); moderately active (70%>SA≥50%); weakly active (50%>SA≥30%) and inactive (SA<30%).

Micro-organisms and Media

Antibacterial activity was determined against certified strains of three micro-organisms, namely *Bacillus cereuss* (Gram-positive), *Escherichia coli* and *Klebsiella pneumoniae* (Gram-negative), which were cultured in the appropriated broths at 30°C for overnight. They were obtained from the Pathology Centre, Sultanah Nur Zahirah Hospital, Kuala Terengganu, Terengganu. The bacterial stock cultures were maintained on the nutrient agar (NA) respectively, which were stored at 28°C.

Antibacterial Activity Assay

The agar cultures of the test micro-organisms were prepared as described previously by Mackeen *et al.* (1997). For an initial screening, 1 mg of compound was loaded onto each Whatman No.1 filter paper disc (∅ 6 mm) and placed on the previously inoculated agar. The plates were inverted and incubated for 24 hour at 30°C. For this purpose, streptomycin and gentamycin were used as positive controls. A stock solution of compound was made in DMSO. The presence of antibacterial activity would be indicated by the occurrence of clear inhibition zones around the disc. The assay was carried out in triplicates. The strength of the activity was classified as strong for inhibition zone diameters (i.d.) >16.0 mm, moderate (good) for diameters ranging from 11 to 16 mm, weak for diameters 7 to 11 mm and no activity for diameters < 7 mm. The diameters (mm) of the growth inhibition halos were measured using a ruler.

Cytotoxic Assay

Cytotoxic assay was carried out against Human Caucassian Promyelocytic leukaemia cells (HL-60 cell line) obtained from the National Cancer Institute, USA. The cells were maintained in

RMPI-1640 medium supplemented with 10% foetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin. The cytotoxic effect of the compound was assessed using the MTT Assay. Briefly, 100µl of exponentially growing HL-60 cells, at the concentration of $1x10^5$ cells/ml, were seeded into 96-flat bottom microwell plate in the presence of various concentrations of compound and incubated for 3 days. A volume of 20µl MTT solution (5mg/ml in PBS) was added to each well. After 4 hours of incubation, the medium was replaced with 100µl of DMSO. The MTT-formazan product, dissolved in DMSO, was estimated by measuring the absorbance at 570nm in the ELISA plate reader (BIO-TECH INSTRUMENT, INC). The cytotoxicity was expressed as inhibition concentration fifty percent (IC_{50}), i.e. the concentration to reduce the absorbance of the treated cells by 50% with reference to the control (untreated cells).

RESULTS AND DISCUSSIONS

Homogenized *Xestospongia* sp. (8 kg, dry weight) was extracted with methanol. The extract (394 g) was then subjected to solvent partitioning, i.e., aq. MeOH against hexane, ethyl acetate and butanol. The extracted hexane (76.34 g) was chromatographed using silica gel Merck Kieselgel 60 PF_{254} Art no. 7749, eluted with hexane/$CHCl_3$/EtOAc. Sequential CC on silica gel led to the isolation of fatty acid compound, octacosanoic acid (*Fig. 1*).

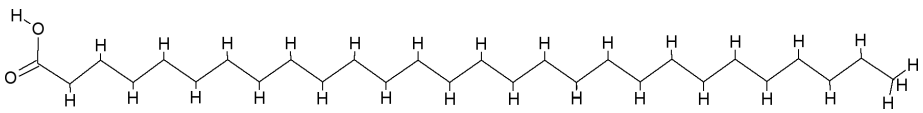

Fig. 1: The octacosanoic acid

Octacosanoic acid (*Fig. 1*) was isolated as a colourless amorphous solid and showed a molecular ion peak at m/z 424 $(M)^+$ in the EIMS. The presence of 28 carbon signals in the ^{13}C NMR spectrum was consistent with the molecular formula of $C_{28}H_{52}O_2$. The data of ^1H NMR and ^{13}C NMR are summarized in Table 1. The characterization of 1 was as follows:

Octacosanoic acid (1). IR (CCl_4) 3649, 2918, 2848, 1702, 1464, 1432, 1410, 1297 cm^{-1}; ^1H-NMR ($CDCl_3$) 0.864 (brt, J =, 3H, -CH_3), 1.265 (brs, 26H, -CH2-), 1.644 (m, 2H), 2.358 (t, 2H); MS, m/e (relative intensity) 424 (M^+), 410 (19), 354 (7), 326 (17), 311 (24), 297 (36), 283 (31), 269 (21), 255 (21), 241 (33), 227 (29), 213 (24), 199 (32), 185 (51), 171 (43), 157 (49), 143 (44), 129 (71), 87 (100).

TABLE 1
^1H-(400MHz) and ^{13}C-(100 MHz) NMR chemical shifts ($CDCl_3$) of octacosanoic acid

Position C	Octacosanoic acid	
	δH	δC
1		178.572
2	2.358 (2H, t)	33.965
3	1.644 (2H, m)	24.929
4-21	1.265 (26H, br, s)	29.926-30.412
22		32.194
23		22.929
24		27.323
25		37.336
26		32.988
27		19.959
28	0.864 (3H, t)	14.351

Three new brominated fatty acids, (5*E*, 11*E*, 15*E*, 19*E*)-20-bromoeicosa-5,11,15,19-tetraene-9,17-diynoic acid, (5Z, 11*E*, 15*E*, 19E)-6,20-dibromoeicosa-5,11,15,19-tetraene-9,17-diynoic acid and (Z *E*)-14,14-dibromo-4,6,13-tetradecatrienoate (characterized as the methyl ester) were isolated from *Xestospongia* sp. from the Indian Ocean (Brantley *et al.*, 1995).

The bioassay results for the isolated compound are shown in Table 2. The octacosanoic acid was inactive in free radical-scavenging activity exhibited only 10.03% inhibition. The growth-inhibition, induced in cancer cells after exposure to the compound, was expressed as the percentage of the cell viability observed in the treated cells versus untreated HL-60. The results are summarized in Table 2. When tested *in vitro* against Human Caucassian Promyelocytic Leukaemia (HL-60) cell line, octacosanoic acid demonstrated cytotoxicity at IC_{50} 2.2 µg/ml, respectively.

In this study, octacosanoic acid was found to give a weak activity against *Bacillus cereuss* and *Escherichia coli* when tested for antimicrobial activity. Only *Klebsiella pneumoniae* inhibited significantly moderate activity by this isolated compound. These results are also presented in Table 2.

TABLE 2
Activities of pure compound isolated from *Xestospongia* sp.

Specimen		[a]AO (%)	[b]Cytotoxic	[c*] Antibacterial		
				B. cereus	*E. coli*	*K. pneumoniae*
Compound	Octacosanoic acid	10.03	2.2	+	+	++
Control	Gentamycin			+++	+++	+++
	Streptomycin			+++	+++	+++

** B.cereus: Bacillus cereuss*; E.coli: *Escherichia coli; K. pneumoniae: Klebsiella pneumoniae*
(-) No activity, (+) weak activity (7-11 mm halo), (++) moderate activity (11-16 mm halo), (+++) high activity (>16 mm halo).
The experiments were run in triplicates. [a]The reduction of the DPPH free radical was measured by reading the UV absorbance at 517 nm. [b]The cytotoxic effect of compound human caucassian promyelocytic leukaemia cells (HL-60 cell line) was assessed by MTT Assay. [c]Antibacterial activity of Octacosanoic acid as measured by the disc diffusion method. The inhibition zones were measured in mm.

CONCLUSIONS

The isolation of hexane extract, from *Xestospongia* sp., yielded a long chain saturated fatty acid compound, i.e. octacosanoic acid, which might not have been reported previously in *Xestospongia*. For its bioactivities, the compound was found to be inactive against antioxidant activity (DPPH), moderate activity against *Klebsiella pneumoniae* in antibacterial activity and strong cytotoxicity against Human Caucassian Promyelocytic Leukaemia (HL-60) cell line. This showed that the Malaysian water is a potential source of sponge secondary metabolites worthy as a lead compound for developing a therapeutic agent for various kind of diseases.

ACKNOWLEDGEMENTS

The authors acknowledge the support from the Ministry of Science, Technology and Innovation for the fund provided (IRPA 01-02-12-0068-EA 10707) and FRGS (vote: 59004). The authors also thank the Department of National Marine Parks for the sampling permit.

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